

In the Claims

1. (currently amended) A recombinant herpes simplex virus, ~~characterized in that a DNA sequence is inserted in the genome, wherein said DNA sequence comprising a heterologous nucleotide sequence selected from the group consisting of the sequences represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5, or a homologous sequence thereof.~~
2. (currently amended) The recombinant herpes simplex virus according to claim 1, ~~characterized in that wherein the DNA heterologous nucleotide sequence is inserted at the XbaI site of the UL2 gene or the UL44 gene of the HSV genome.~~
3. (currently amended) The recombinant herpes simplex virus according to claim 1, ~~characterized in that wherein the DNA heterologous sequence represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5 is inserted in either a nonessential gene regions-region of the HSV genome.~~
4. (currently amended) A method for the production of a recombinant herpes simplex virus, comprising ~~constructing obtaining at least one DNA segments represented by segment represented by a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 or, and SEQ ID NO: 5 respectively;~~ and inserting the ~~five~~ DNA segments-segment into ~~an~~ HSV genome using gene engineering method(s), respectively, thereby obtaining the recombinant herpes simplex virus.
5. (canceled).

2) adding solid sodium chloride to the cell lysis solution until the final concentration is 1.0 to 1.2 mol/L with stirring for dissolution, then centrifugating the mixture and leaving the supernatant;

3) precipitating rAAV with PEG/NaCl, adding solid polyethylene glycol to the sodium chloride-containing supernatant of step 2) with stirring for dissolution, letting the mixture sit, then centrifugating the mixture and discarding the supernatant but leaving the precipitate;

4) treating the cell lysis solution with DNaseI and RNase to degrade the nucleic acid, dissolving the precipitate of step 3), and adding DNaseI and RNase to dissolve the residual nucleic acid apart from the rAAV viral particles;

5) using chloroform to extract and remove other proteins and the residual PEG, adding chloroform to extract, and then, centrifugating the mixture and removing the upper water phase;

6) removing salts via dialysis; and

7) further purifying rAAV via density gradient centrifugation or affinity chromatography.

8. (currently amended) A recombinant vector plasmid pSNAV-NX, characterized in that the recombinant plasmid comprises said plasmid comprising an ITRs at the each of the two ends of an AAV genome selected from the group consisting of the AAV-1, AAV-3, AAV-4, AAV-5 or and AAV-6 genomes; and further comprising, with an immediate early enhancer and a promoter of cytomegalovirus, a multiple cloning site and a polyA signal successively intervening between the two ITRs, and a neomycin resistant gene expression cassette flanking the outside of at least one of the ITRs.

6. (currently amended) A method for large-scale production and preparation of a recombinant adeno-associated virus serotype 1, 3, 4, 5, or 6, characterized in that the method comprises comprising the steps of:

- (1) preparing and producing the obtaining a recombinant herpes simplex viruses virus comprising a heterologous nucleotide sequence selected from the group consisting of the sequences represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5 according to claim 1;
- (2) establishing "a vector cell", namely, a recombinant AAV vector cell strain;
- (3) infecting corresponding the vector cell strains respectively with the five recombinant herpes simplex viruses of (1); and
- (4) producing a lot of culturing the infected vector cell strain under conditions whereby recombinant adeno-associated viruses are produced by vector cell strains infected respectively with the five recombinant herpes simplex viruses.

7. (currently amended) A method for isolation and purification of recombinant adeno-associated virus serotype 1, 3, 4, 5 or 6, characterized in that a crude lysis solution comprising recombinant adeno-associated virus containing cells and the culture medium thereof is isolated and purified via the following steps:

- 1) adding chloroform to the a crude lysis solution comprising recombinant adeno-associated virus-containing cells to deactivate any HSV helper viruses, lyse cells, and denature and precipitate a great many cell proteins to obtain cell lysis solution;

9. (currently amended) The method for isolation and purification of of claim 7 wherein the recombinant adeno-associated virus has a serotype selected from the group consisting of serotypes 1, 3, 4, 5, and 6 according to claim 7, characterized in that the method is useful for large scale isolation and purification of the so-called "AAV empty capsid", i.e. AAV serotype 1, 3, 4, 5 or 6 without gene therein.

10. (currently amended) A method for purification of recombinant adeno-associated virus serotype 1, 3, 4, 5, or 6, characterized in that the method comprisescomprising: obtaining a solution containing rAAV of serotype 1, 3, 4, 5, or 6; adjusting the conductance value of obtained rAAV solution before passing through an ion exchange column which has been balanced by a buffer, balancing the ion exchange column using a buffer again, then; eluting the ion exchange column with a salt-containing buffer and collecting the elution peaks; passing the collected elution peaks through a molecular sieve column which has been balanced by a buffer, followed by; and washing the column with a buffer again.

11. (new) The method of claim 7 wherein the rAAV is an empty capsid of an AAV serotype selected from the group consisting of serotypes 1, 3, 4, 5, and 6.